Ethnicity as a determinant of ovarian reserve: differences in ovarian aging between Spanish and Indian women

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Objective: To investigate differences in ovarian reserve markers (antimüllerian hormone [AMH] and antral follicle count [AFC]) in Indian and Spanish women.
Design: Cross-sectional study.
Setting: In vitro fertilization (IVF) clinics.
Patient(s): Infertile Spanish (n = 229) and Indian (n = 236) women who underwent controlled ovarian stimulation for IVF from January to October 2012.
Intervention(s): None.
Main Outcome Measure(s): Data on ovarian reserve markers and results after ovarian stimulation were collected.
Result(s): The mean age of women undergoing their first or second IVF cycle was significantly higher in Spanish than in Indian women (37.5 ± 3.3 years vs. 31.5 ± 3.8 years). Despite this 6-year age gap, AFCs were similar (9.5 ± 4.7 vs. 9.9 ± 4.6), as were day 3 FSH levels (7.5 ± 4.5 IU/L vs. 6.9 ± 2.3 IU/L). AMH levels were slightly lower in Spanish women (1.6 ± 1.7 ng/mL vs. 2.5 ± 1.6 ng/mL). Multivariate regression analysis showed that being Indian decreased AFC by 2.3, such that AFC in Indian women was similar to that in Spanish women 6.3 years older (95% confidence interval 3.39–1.10).
Conclusion(s): Similar ovarian reserve markers and ovarian response were observed in women with a 6-year age difference in favor of the Spanish, suggesting ethnic differences in ovarian aging. Further research is needed to understand whether these differences are genetically induced or are caused by other variables, such as nutrition. Our results may help clinicians to counsel infertile women when discussing assisted reproductive technology outcomes according to age and ethnic background. (Fertil Steril® 2014;102:244–9. ©2014 by American Society for Reproductive Medicine.)
Key Words: Ethnicity, ovarian aging, AMH, AFC

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There is growing evidence that female reproductive function may differ by race. Ethnicity strongly influences the prevalence of several gynecologic diseases. For example, patients of South Asian Indian descent have higher rates of insulin resistance and polycystic ovary syndrome (1). Puberty onset has been reported to start earlier in Asian American girls than in white American girls (2). Considering the increasing demand for assisted reproductive technology (ART) as well as the strong influence of ovarian reserve on ART outcome, it might be found that ovarian reserve may also differ according to ethnic origins. This difference may have significant implications when evaluating ovarian reserve to categorize patients and to establish prognoses for ovarian response, thus avoiding excessive response or prescribing adequate protocols for suspected poor responders.

Ethnicity has been consistently shown to affect ART outcome (3–8). Some ethnic groups (Asian, African American, and Hispanic) have significantly lower clinical pregnancy and live birth rates and higher miscarriage rates after ART than whites (6). Despite younger age and similar embryo quality, Indian American women had a significantly lower live birth rate than white American women in an earlier investigation (9).

Earlier studies have focused on basal hormonal fluctuations in various

ethnic populations. Day 3 FSH levels were higher in African American women than in age-matched white women (10–12). Similarly, antimüllerian hormone (AMH) levels were lower in African-American and Hispanic women (25.2% and 24.6% lower, respectively) than in white women (13). Thus, there may be an independent effect of race and ethnicity on the age-related declines in AMH levels and ovarian function over time.

The purpose of the present study was to investigate whether there were any differences in ovarian reserve markers (AMH and antral follicle count [AFC]) between age-matched populations of Indian and Spanish women.

MATERIALS AND METHODS

Experimental Design

We conducted a prospective cohort study of 465 infertile Spanish and Indian women who underwent controlled ovarian stimulation (COS) for in vitro fertilization (IVF) from January to October 2012 in IVF centers in Spain and India. The population included prospectively in this study consisted of 229 infertile Spanish women and 236 infertile Indian women undergoing their first or second IVF cycles. Spanish patients were monitored at IVI Madrid, and Indian patients were followed at Nova IVI Fertility clinics in Delhi and Ahmedabad.

Inclusion and Exclusion Criteria

To include a homogeneous population, the criteria for inclusion in this study were age ≤42 years, both ovaries present, and undergoing IVF treatment because of male-factor infertility, tubal disease, or previous failed intrauterine insemination. Women diagnosed with polycystic ovary syndrome according to the Rotterdam criteria, endometriosis stage III–IV, previous adnexal surgery, or pelvic inflammatory disease were excluded.

Patients provided written informed consent, and Institutional Review Board (IVI-Madrid) approval from our institution was obtained before initiation of the study (MAD-CI-11-2012-01). Data were gathered anonymously to avoid individual patient identification, as consistent with data protection rules in our institution.

Treatment Protocol

Patients underwent conventional COS as previously described (14). Briefly, patients received a starting dose of recombinant FSH (Puregon, Organon; or Gonal F, Serono) or highly purified hMG (Menopur, Ferring) ranging from 150 IU to 225 IU; 0.25 mg GnRH antagonist Ganirelix (Orgalutran, Organon) was administered daily starting on day 5 or 6. The cycle was monitored according to the policy of the patient's clinic. Recombinant hCG (Ovitrelle, Serono) was administered as soon as two leading follicles reached ≥17 mm mean diameter, and oocyte pickup was performed 36 hours later. Fertilization was performed with conventional IVF or intracytoplasmic sperm injection (ICSI), according to individual criteria.

Data collected included age, body mass index (BMI), ovarian reserve markers (day 3 serum FSH, and E_2, and AMH levels), and AFC via transvaginal ultrasonography. We also collected IVF results after COS for both ethnic groups, including the amounts of gonadotropin used, the number of oocytes retrieved, the number of mature oocytes, the number of oocytes fertilized, and the number of embryos obtained.

AMH levels were measured in patient serum. Blood was collected by peripheral venipuncture; the serum was separated from cells by centrifugation, and samples were frozen at −20°C until assayed. Immunoreactive AMH concentrations were determined with the use of ELISA (Immunotech; Beckman Coulter) according to the manufacturer's instructions. All samples were tested in the same assay and performed in duplicate. The intra-assay and interassay coefficients of variation were 12.3% and 14.2%, respectively (15).

We followed the recommendations of Brokemans et al. (16) to correctly measure antral follicles. With the use of a real-time two-dimensional transvaginal transducer, we counted follicles from days 2 to 4 of the cycle to avoid the effect of intracycle variation. Our counts included all antral follicles 2–10 mm in diameter.

We used the following systematic process to count antral follicles: 1) Identify the ovary; 2) explore the dimensions in two planes (perform a scout sweep); 3) decide on the direction of the sweep to measure and count follicles; and 4) measure the largest follicle in two dimensions. If the largest follicle was ≤10 mm in diameter, we started to count from the outer ovarian margin of the sweep to the opposite margin, considering every round or oval transonic structure within the ovarian margins to be a follicle. We repeated this procedure with the contralateral ovary, and combined the number of follicles in each ovary to obtain the AFC. If the largest follicle was >10 mm in diameter, we further ascertained the size range of the follicles by measuring each sequentially smaller follicle until a follicle with a diameter ≤10 mm was found. We then performed a total count (as described) regardless of follicle diameter, and subtracted the number of follicles >10 mm from the total follicle count to determine the AFC (16).

Statistical Analysis

Because no previous data existed to take as a reference for our sample size calculations, we decided to evaluate 200 patients per arm, considering that the data obtained from these women would be clinically relevant.

Categoric data are expressed as number and percentage, and continuous data are expressed as mean ± SD. Continuous variables were examined for normality with the Kolmogorov-Smirnov test; if the data were not normally distributed, nonparametric tests were used. Statistical analyses were performed with the chi-square test, Fisher exact test, or two-sample Student t test. Linear regression analysis was used to assess the association between variables. To control for confounding factors, stratification was undertaken for: cause of infertility, age, BMI, and duration of infertility. Factors having an impact on ovarian reserve were assessed with the use of a multivariate analysis that included the following potential related factors: age, AFC, AMH, basal FSH, and number of

oocytes. Significance was set at $P<.05$. Statistical calculations were performed on an intention-to-treat basis with the use of SPSS 13.0.

RESULTS

A total of 465 patients met the inclusion criteria and participated in the study: 229 Spanish (49.2%) and 236 Indian (50.8%). The mean age of women undergoing their first or second IVF cycles was significantly higher in Spanish patients than in Indian patients (37.5 ± 3.3 years vs. 31.5 ± 3.8 years, respectively; $P<.001$). BMI was significantly lower in Spanish women than in Indian women (22.1 ± 3.1 kg/m^2 vs. 24.9 ± 4.3 kg/m^2, respectively; $P<.001$). Demographic characteristics for both groups are summarized in Table 1.

Interestingly, even though the groups differed in mean age, AFCs were similar for the two populations of different ethnic origin (9.5 ± 4.7 for Spanish vs. 9.9 ± 4.6 for Indians; $P=.4$). Day 3 FSH levels also were similar between the groups (7.9 ± 4.5 IU/L vs. 6.9 ± 2.3 IU/L; $P=.002$). Lower AMH levels were detected in Spanish patients than in Indian patients (1.6 ± 1.7 ng/mL vs. 2.5 ± 1.6 ng/mL, respectively; $P=.03$).

IVF/ICSI cycle outcomes were similar for the two patient populations, despite the age gap between Indian and Spanish women. Similar numbers of total oocytes (8.8 ± 6.9 vs. 8.8 ± 5.6; $P=.9$) and metaphase II oocytes (6.7 ± 4.9 vs. 7.5 ± 4.8; $P=.14$) were retrieved. Even though similar numbers of oocytes were retrieved, Indian patients received a significant higher amount of gonadotropins than Spanish patients (2,910 ± 902 IU vs. 2,420 ± 840 IU, respectively; $P<.001$).

We conducted univariate linear regression analyses to assess the effect of ethnic origin on ovarian reserve markers. As expected, there was a significant negative correlation between age and AFC/AMH levels (Indian patients: age/AFC: $r = -0.2503$; $P=.0103$; age/AMH: $r = -0.1513$; $P=.0257$; Spanish patients: age/AFC: $r = -0.2834$; $P<.0001$; age/AMH: $r = -0.2119$; $P<.001$). This association reflected the 6-year age gap between ethnic groups. We observed, unsurprisingly, that as maternal age increased, both AFC and serum AMH levels diminished (Figs. 1 and 2).

To further analyze the association between variables involved in ovarian reserve and ethnicity, multivariate logistic regression was performed. Younger age, higher BMI, lower AMH, higher FSH, and longer duration of infertility were all associated with Indian ethinicity ($P<.0001$ for all). Multivariate analysis allowed us to investigate whether these confounding variables were masking the relationship between AFC and ethnicity. Being Indian decreased AFC by 2.3 (95% confidence interval [CI] 3.39–1.10), such that AFC in Indians patients was similar to Spanish patients 6.3 years older.

DISCUSSION

The data obtained in this study indicate that significant differences exist between Spanish and Indian women regarding ovarian reserve markers. Our data further suggest that ethnicity may be important when considering age as a prognostic factor in ART. Although the Indian women in our study population were younger than the matched white Spanish women, they had similar ovarian reserve markers, suggesting a 6-year advancement in ovarian aging. The fact that AFC and day 3 FSH levels were similar but AMH was significantly lower in Indian women—despite being 6 years younger than the Spanish women—may reflect differences in early diagnostic capacity of diminished ovarian reserve.

Race-dependent differences in the reproductive life cycle have been reported. Puberty has been reported to start earlier for African-American children than for white children [17]. There is controversy as to whether Asian children mature earlier than white children; many authors support an earlier start, although others report a later beginning [2]. Although environmental endocrine disruptors may accelerate the timing of puberty onset, genetic regulation of puberty has been reported to explain at least 50% of the variability in timing [18].

Regarding the last stages of reproductive aging, nonwhite American women were associated with a significantly lower median age at menopause than white American women (49.3 years vs. 51.2 years, respectively; $P<.05$). Furthermore, time to natural menopause was four times faster in blacks than in whites, indicating that black women enter menopause 2 years earlier than white women [19].

After adjusting for many cofounders, there is a consistent finding of race as a risk factor for poor ART outcomes. Sharara and McClamrock reviewed multiple cycles per woman studied and concluded that black women experienced lower implantation and clinical pregnancy rates per cycle than white women (9.8% vs. 23.4%, respectively; $P=.009$), and that the ongoing pregnancy rate per cycle was significantly lower in black women than in white women (14.9% vs. 38.8%, respectively; $P<.005$) [3].

Seifer et al. [13] reported that infertile black women treated for the first time were 31% less likely to achieve a live birth than white women, and that those who had never had an earlier ART treatment were 33% less likely than white women to achieve a live birth ($P<.001$). They also reported 10% lower adjusted odds of live birth after transfer of cryopreserved embryos in black women compared with white women [4]. In a related line of investigation, that group recently published that blacks and Hispanics have an earlier puberty than whites, and that all races have significantly lower birth rates after ART compared with whites [5]. They
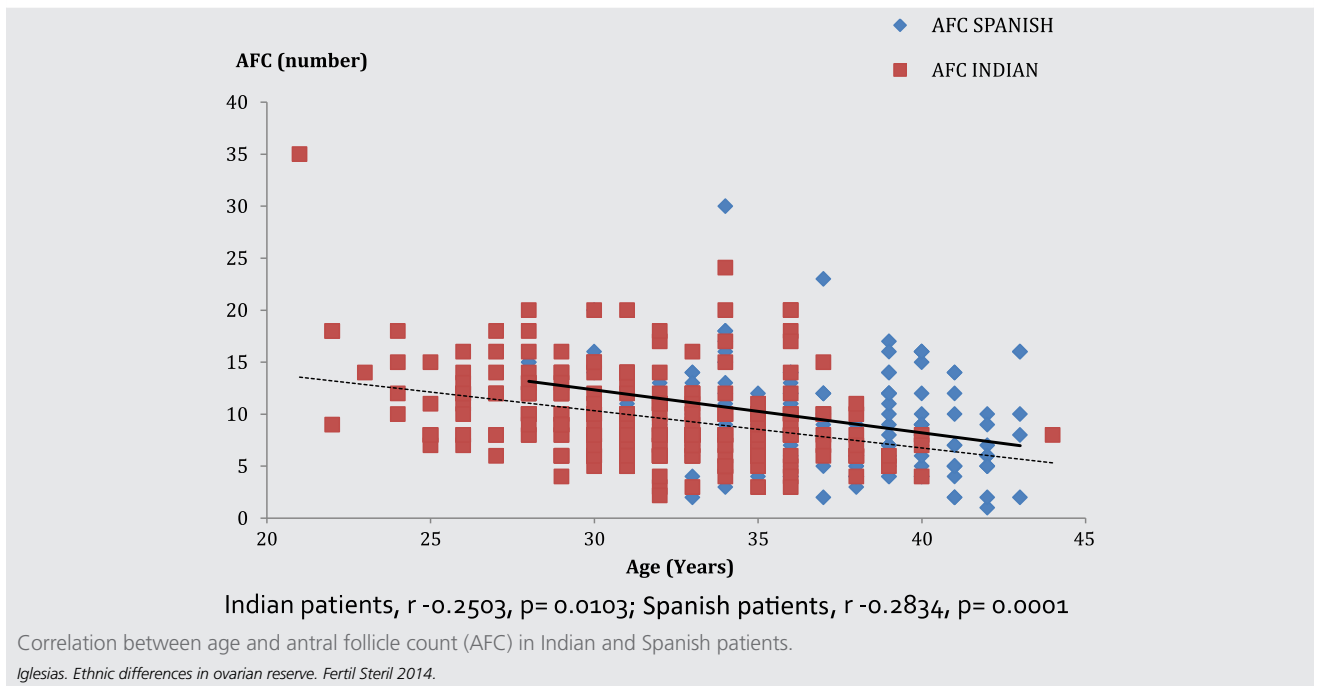
TABLE 1

Demographic data for the study population.

	Spanish	Indian	P value
n	229	236	
Age (y)	37.5 ± 3.3	31.5 ± 3.8	<.0001
Average BMI (kg/m^2)	22.1 ± 3.1	24.9 ± 4.3	<.0001
AMH (ng/mL)	1.6 ± 1.7	2.5 ± 1.6	.03
FSH, day 2–3 (IU/L)	7.9 ± 4.5	6.6 ± 2.3	.002
E$_2$, day 2–3 (pg/mL)	55.7 ± 40.3	41.4 ± 20.2	<.0001
AFC	9.5 ± 4.7	9.9 ± 4.6	.4
Days of stimulation	10.1 ± 2.2	9.5 ± 1.4	.0004
Oocytes retrieved	8.8 ± 6.9	8.8 ± 5.6	.9
Metaphase II oocytes retrieved	6.7 ± 4.9	7.5 ± 4.8	.14
Fertilized oocytes	5.2 ± 3.9	6.2 ± 4.4	.008

Note: AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index.

Iglesias. Ethnic differences in ovarian reserve. Fertil Steril 2014.

FIGURE 1

Indian patients, r -0.2503, p= 0.0103; Spanish patients, r -0.2834, p= 0.0001

Correlation between age and antral follicle count (AFC) in Indian and Spanish patients.

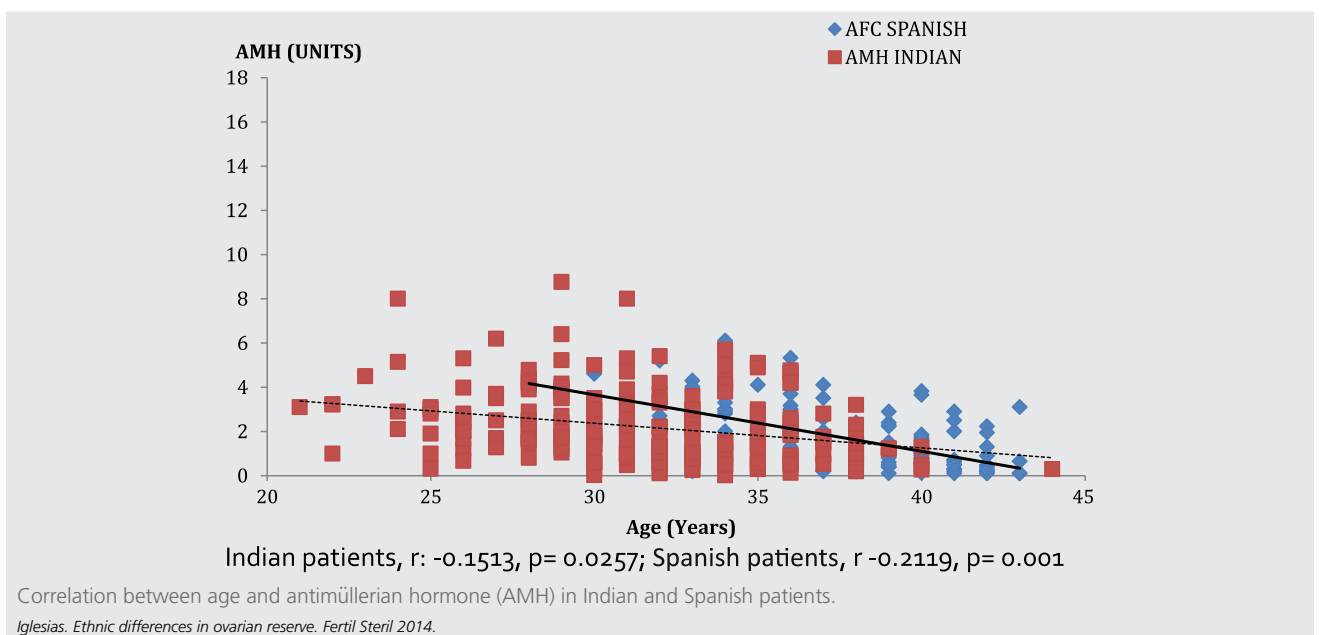*Iglesias. Ethnic differences in ovarian reserve. Fertil Steril 2014.*

also compared outcomes after ART in 2004–2006 with previously reported outcomes in 1999–2000, concluding that the proportion of live births per cycle increased across all groups over time, with greater increases occurring for white women. Fujimoto et al. compared ART outcomes among white, black, Asian, and Hispanic women and concluded that all ethnic groups had significantly lower clinical pregnancy rates, lower live birth rates after ART, and higher miscarriage/stillbirth rates than white women (*P*<.001) (6).

Purcell et al. determined whether success rates were similar in Asian and white women undergoing infertility treatment, and reported that Asian women had a decreased clinical pregnancy rate (odds ratio 0.71, 95% CI 0.64–0.80) and a decreased live birth rate (odds ratio 0.69, 95%

FIGURE 2

Indian patients, r: -0.1513, p= 0.0257; Spanish patients, r -0.2119, p= 0.001

Correlation between age and antimüllerian hormone (AMH) in Indian and Spanish patients.

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confidence interval 0.61–0.77); they concluded that after treatment, infertile Asian women had significantly fewer pregnancies than white women [7]. In a recent retrospective study, Jayaprakasan et al. [8] confirmed that ethnicity is a major determinant of live birth following IVF treatment, with lower live birth rates in southeast Asian, black African, and Middle-Eastern women than in white European women.

All of these reports investigated live birth rate after IVF-ICSI, an event that may be influenced by many confounding variables, including genetic determinants of quantitative and/or qualitative ovarian reserve, endometrial receptivity, lifestyle and environmental factors, and socioeconomic status. No information on male partner ethnicity was provided in these studies. The present study focused on clinical markers of ovarian reserve, because if true biologic differences exist, they may profoundly affect patient counseling and the adaptation of COS protocols to biologic age rather than chronologic age.

Whether these differences are genetically based is still under investigation. Schuh-Huerta et al. sought to identify genetic variants associated with ovarian reserve, hypothesizing that follicle number and menopausal age share underlying genetic associations [20]. They found that variant RS 16991615 in the gene encoding MCM8 was significantly associated with a later menopausal age of $+1.07 \pm 0.11$ years in previous studies, and greater follicle numbers of $+2.79 \pm 1.67$ follicles in the white cohort of their study [20].

Variations in ovarian aging patterns have recently been associated with specific ovarian genotypes and subgenotypes of the gene encoding FMR1 as well. Reduced ovarian reserve was previously associated with a shift toward an increased number of CGG repeats [21]. Gleicher et al. investigated whether disparities between races were associated with differences in the distributions of *FMR1* genotypes and subgenotypes, concluding that functional ovarian reserve changes in different races at different rates, an effect that parallels ovarian *FMR1* genotypes and subgenotype distributions [22]. The gene encoding NOBOX may influence gonadotropin-independent follicle development, because women with premature ovarian insufficiency show a high prevalence of mutations in this gene [23].

Each stage of the reproductive life cycle is sensitive to genetic and environmental influences that may correlate with race and ethnicity [24]. Data from the present study show that ovaries from Indian women age 6 years earlier than white Spanish women. Ethnicity should be considered to be a risk factor for diminished ovarian reserve, because we observed that ART outcomes were less optimal in a population of infertile Indian women compared with white Spanish women. Our findings may help clinicians to adequately counsel infertile women about fertility options according to their age and ethnic background, because biologic differences may require medical assistance at earlier stages. Further research is needed to understand whether these differences are genetically determined or whether other contributing factors, such as environment, nutrition, or even lifestyle factors, such as sun exposure–related vitamin D deficiency, play a role.

REFERENCES

1. Rodin DA, Bano G, Bland JM, Taylor K, Nussey SS. Polycystic ovaries and associated metabolic abnormalities in Indian subcontinent Asian women. Clin Endocrinol (Oxf) 1998;49:91–9.
2. Weaver CM, McCabe LD, McCabe GP, Novotny R, van Loan M, Going S, et al. Bone mineral and predictors of bone mass in white, Hispanic, and Asian early pubertal girls. Calcif Tissue Int 2007;81:352–63.
3. Sharara FI, McClamrock HD. Differences in in vitro fertilization (IVF) outcome between white and black women in an inner-city, university-based IVF program. Fertil Steril 2000;73:1170–3.
4. Seifer DB, Zackula R, Grainger DA. Trends of racial disparities in assisted reproductive technology outcomes in black women compared with white women: Society for Assisted Reproductive Technology 1999 and 2000 vs. 2004–2006. Fertil Steril 2010;93:626–33.
5. Butts S, Seifer D. Racial and ethnic differences in reproductive potential across the life cycle. Fertil Steril 2010;93:681–90.
6. Fujimoto VY, Luke B, Brown MB, Jain T, Armstrong A, Grainger DA, et al. Racial and ethnic disparities in assisted reproductive technology outcomes in the United States. Fertil Steril 2010;93:382–90.
7. Purcell K, Schembri M, Frazier LM, Rall MJ, Shen S, Croughan M, et al. Asian ethnicity is associated with reduced pregnancy outcomes after assisted reproductive technology. Fertil Steril 2007;87:297–302.
8. Jayaprakasan K, Pandian D, Hopkisson J, Campbell B, Maalour W. Effect of ethnicity on live birth rates after in vitro fertilisation or intracytoplasmic sperm injection treatment. BJOG 2014;121:3000–7.
9. Shahine LK, Lamb J, Lathi R, Milki A, Langen E, Westphal L. Poor prognosis with in vitro fertilization in Indian women compared to Caucasian women despite similar embryo quality. PLoS One 2009;4:e7599.
10. Randolph JF Jr, Sowers M, Gold EB, Mohr BA, Luborsky L, Santoro N, et al. Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status. J Clin Endocrinol Metab 2003;88:1516–22.
11. Freeman EW, Sammel MD, Gracia CR, Kapoor S, Lin H, Liu L, et al. Follicular phase hormone levels and menstrual bleeding status in the approach to menopause. Fertil Steril 2005;83:383–92.
12. Manson JM, Sammel MD, Freeman EW, Grisso JA. Racial differences in sex hormone levels in women approaching the transition to menopause. Fertil Steril 2001;75:297–304.
13. Seifer DB, Golub ET, Lambert-Messerlian G, Benning L, Anastos K, Watts DH, et al. Variations in serum müllerian inhibiting substance between white, black, and Hispanic women. Fertil Steril 2009;92:1674–8.
14. Garcia-Velasco JA, Bermejo A, Ruiz F, Martinez-Salazar J, Requena A, Pellicer A. Cycle scheduling with oral contraceptive pills in the GnRH antagonist protocol vs the long protocol: a randomized, controlled trial. Fertil Steril 2011;96:590–3.
15. Oriol B, Barrio A, Pacheco A, Serna J, Zuzuarregui JL, Garcia-Velasco JA. Systemic methotrexate to treat ectopic pregnancy does not affect ovarian reserve. Fertil Steril 2008;90:1579–82.
16. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. Fertil Steril 2010;94:1044–51.
17. Koprowski C, Ross RK, Mack WJ, Henderson BE, Bernstein L. Diet, body size and menarche in a multiethnic cohort. Br J Cancer 1999;79:1907–11.
18. Palmert MR, Boepple PA. Variation in the timing of puberty: clinical spectrum and genetic investigation. J Clin Endocrinol Metab 2001;86:2364–8.
19. Bromberger JT, Matthews KA, Kuller LH, Wing RR, Meilahn EN, Plantinga P. Prospective study of the determinants of age at menopause. Am J Epidemiol 1997;145:124–33.
20. Schuh-Huerta S, Johnson N, Rosen M, Sternfeld B, Cedars M, Pera R. Genetic markers of ovarian follicle number and menopause in women of multiple ethnicities. Hum Genet 2012;131:1709–24.
21. Karimov CB, Moragianni VA, Cronister A, Srouji S, Petrozza J, Racowsky C, et al. Increased frequency of occult fragile X–associated primary ovarian insufficiency in infertile women with evidence of impaired ovarian function. Hum Reprod 2011;26:2077–83.

22. Gleicher N, Kim A, Weghofer A, Barad D. Differences in ovarian aging pattern between races are associated with ovarian genotypes and sub-fenotypes of the FMR1 gene. Reprod Biol Endocrinol 2012;10:77.

23. Murray A, Bennett CE, Perry JR, Weedon MN, Jacobs PA, Morris DH, et al, Reprogen Consortium. Common genetic variants are significant risk factors for early menopause: results from the Breakthrough Generations Study. Hum Mol Genet 2011;20:186–92.

24. Tal R, Seifer DB. Potential mechanisms for racial and ethnic differences in antimullerian hormone and ovarian reserve. Int J Endocrinol 2013;2013. Epub 2013 Nov 21. http://dx.doi.org/10.1155/2013/818912.